HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

Pharmacological Inhibition of the Chemokine C-C Motif Chemokine Ligand 2 (Monocyte Chemoattractant Protein 1) Accelerates Liver Fibrosis Regression by Suppressing Ly-6C$^+$ Macrophage Infiltration in Mice

Christer Baeck,[1] Xiao Wei,[1] Matthias Bartneck,[1] Viktor Fech,[1] Felix Heymann,[1] Nikolaus Gassler,[2] Kanishka Hittatiya,[3] Dirk Eulberg,[4] Tom Luedde,[1] Christian Trautwein,[1] and Frank Tacke[1]

Macrophages constitute a major proinflammatory component during chronic liver diseases and are considered a key factor in promoting hepatic fibrosis. However, there is increasing evidence that distinct monocyte and macrophage subsets exert critical functions in regression from organ fibrosis as well. Experimental mouse models of fibrosis regression have identified "restorative" macrophages as Ly-6C (Ly6C, Gr1) low-expressing, monocyte-derived cells. We investigated molecular pathways balancing proinflammatory and restorative macrophages during fibrosis regression as well as pharmacologically augmenting beneficial macrophage functionality in fibrosis resolution. Therefore, we employed a Spiegelmer-based inhibitor of the chemokine, C-C motif chemokine ligand 2 (CCL2; monocyte chemoattractant protein 1), termed mNOX-E36, in the regression phase of two murine models of toxic (CCl_4) and metabolic (methionine-choline–deficient diet) liver fibrosis. Although inflammation rapidly declined after cessation of injury, we observed a transient influx of Ly-6C$^+$ infiltrating monocytes (iMΦ), which are characterized by typical macrophage morphology, up-regulated expression of CCR2, and the pro-inflammatory cytokine, tumor necrosis factor (TNF), in injured liver. By inhibiting the early influx of Ly-6C$^+$ iMΦ by the CCL2 inhibitor, mNOX-E36, the intrahepatic macrophage equilibration shifted toward the "restorative" Ly-6C$^-$ subset of iMΦ. Consequently, fibrosis resolution was significantly accelerated upon mNOX-E36 administration in both models. Blocking transient recruitment of infiltrating Ly-6C$^+$ monocytes, but not direct effects of the inhibitor on the remaining macrophages, resulted in reduced intrahepatic levels of proinflammatory cytokines. *Conclusion:* Transient CCL2-dependent recruitment of infiltrating Ly-6C$^+$ monocytes during fibrosis regression counteracts scar resolution by perpetuating inflammatory reactions through release of proinflammatory cytokines such as TNF. Pharmacological inhibition of Ly-6C$^+$ monocyte recruitment using the CCL2-inhibitor, mNOX-E36, accelerates regression from toxic and metabolic liver fibrosis in two independent experimental models. (HEPATOLOGY 2014;59:1060-1072)

Liver fibrosis is a characteristic consequence of chronic organ inflammation. Persistent hepatic inflammation promotes apoptosis of parenchymal cells and replacement by connective tissue and extracellular matrix (ECM) proteins. However, the traditional view of liver fibrosis as an irreversible process is obsolete, because it is now evident that the development of hepatic fibrosis is a dynamic, potentially bidirectional process. Spontaneous resolution of scarring is observed in animal models of liver fibrosis and in

Abbreviations: ALT, alanine aminotransferase activity; AST, aspartate aminotransferase; BM, bone marrow; BMMs, BM-derived macrophages; BW, body weight; CCL, C-C motif chemokine ligand; CCR, C-C motif chemokine receptor; ECM, extracellular matrix; ELISA, enzyme-linked immunosorbent assay; FACS, fluorescence-activated cell sorting; FCS, fetal calf serum; IFN, interferon; IL, interleukin; iMΦ, infiltrating macrophages; IP, intraperitoneally; KCs, Kupffer cells; mAbs, monoclonal antibodies; MCD, methionine-choline deficient; MCP-1, monocyte chemoattractant protein 1; MMP, matrix metalloproteinase; mNOX-E36, murine MCP-1 inhibitor; mRNA, messenger RNA; NK, natural killer; qPCR, quantitative polymerase chain reaction; RFP, red fluorescent protein; SC, subcutaneously; SD, standard deviation; α-SMA, alpha-smooth muscle actin; Tg, transgenic; TG, triglyceride; TNF, tumor necrosis factor; WT, wild type.

human trials in which the stimuli responsible for chronic or repeated hepatic inflammation is successfully removed.[1,2]

Hepatic macrophages, which comprise resident Kupffer cells (KCs) as well as infiltrating monocyte-derived cells, are key promoters of hepatic fibrosis.[3] Inflammatory monocytes and activated macrophages in injured liver release proinflammatory and profibrogenic cytokines, orchestrate the subsequent inflammatory reaction, and directly activate collagen-producing hepatic stellate cells in murine models of liver fibrosis and in human fibrotic liver.[4-6] However, macrophages appear to be important beneficial contributors to fibrosis regression as well. The selective depletion of macrophages, using a transgenic (Tg) mouse model with diphtheria toxin receptor expression on myeloid ($CD11b^+$) cells, during the resolution phase after experimental injury significantly impaired resolution of fibrosis.[7] This effect could be linked to reduced expression of matrix metalloproteinase (MMP)−13 (collagenase 3) by scar-associated macrophages, which is a key enzyme to degrade excessive ECM proteins.[8] Moreover, macrophage-derived MMP-12 (elastase) regulates the degradation of elastin, which is a major ECM component characterizing advanced fibrosis and cirrhosis.[9] In line with this, the adoptive transfer of macrophage subsets or monocyte-derived dendritic cells into (regressing) experimental fibrosis was found to improve hepatic fibrosis in mice.[10,11]

These data collectively indicated the presence of "restorative macrophages" during fibrosis regression, which likely derive from an infiltrating monocyte precursor. By using sophisticated labeling, transfer, and depletion strategies in mice, macrophages with a clear restorative phenotype were recently identified as a low-Ly-6C–expressing (Gr-1) subpopulation ($Ly\text{-}6C^-$) of infiltrating macrophages (iMΦ) in regression from murine toxic liver fibrosis.[1] During scar remodeling, the subsets of iMΦ can undergo a shift from rather immature $Ly\text{-}6C^+$ to more mature $Ly\text{-}6C^-$ macrophages, which displayed increased expression of MMPs, growth factors, and phagocytosis-related genes, including Mmp9, Mmp12, insulin-like growth factor 1, and glycoprotein (transmembrane) nmb.[1]

It is currently unclear by which pathways these restorative macrophages are recruited or regulated or whether augmentation of such beneficial macrophage subsets could be achieved by pharmacological interventions. Because the chemokine receptor C-C motif chemokine receptor 2 (CCR2) was found to be mainly responsible for the recruitment of proinflammatory and profibrogenic infiltrating monocytes during fibrosis progression,[4] we hypothesized that pharmacological inhibition of the chemokine C-C motif chemokine ligand (CCL2), also called monocyte chemoattractant protein 1 (MCP-1), might bear therapeutic potential during fibrosis regression. In the injured liver, CCL2 is synthesised by activated stellate cells, hepatocytes, macrophages, and endothelial cells.[12,13] In patients with fibrosis and cirrhosis, increased hepatic CCL2 expression levels, as well as CCR2-dependent macrophage infiltration, have been described.[5,14] CCR2- or CCL2-deficient mice were shown to be protected from liver fibrosis in experimental models,[4,12,15] but displayed also altered kinetics of regression from injury.[16]

To test our hypothesis, we used a CCL2-inhibiting Spiegelmer. Spiegelmers are short, L-enantiomeric RNA molecules that are developed to specifically bind and inhibit the protein of interest, conceptually similar to monoclonal antibodies (mAbs).[17] In contrast to aptamers, which are composed of natural D-nucleotides or their respective derivates, Spiegelmers are nuclease resistant and therefore biostable as well as immunologically inert through use of non-natural L-nucleotides.[18] The Spiegelmer employed in our studies, mNOX-E36 (murine MCP-1 inhibitor), binds with high affinity to murine CCL2 and inhibits its biological effects at low nanomolar concentrations.

From the [1]Department of Medicine III, University Hospital Aachen, Aachen, Germany; [2]Institute of Pathology, University Hospital Aachen, Aachen, Germany; [3]Institute of Pathology, University Bonn, Bonn, Germany; and [4]NOXXON Pharma AG, Berlin, Germany.

Received April 5, 2013; accepted October 1, 2013.

This work was supported by the German Research Foundation (DFG Ta434/2-1 and DFG SFB/TRR 57) and by the Interdisciplinary Center for Clinical Research (IZKF), Aachen, Germany.

Address repirint requests to: Frank Tacke, M.D., Ph.D., Department of Medicine III, University Hospital Aachen; Pauwelsstraße 30, 52074 Aachen, Germany. E-mail: frank.tacke@gmx.net; fax: +49-241-80-82455.

Copyright © 2014 by the American Association for the Study of Liver Diseases.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.26783

Potential conflict of interest: The antimurine MCP-1 inhibitor, mNOX-E36, was kindly provided by NOXXON Pharma AG. D.E. is an employee of NOXXON Pharma AG (Berlin, Germany).

Additional Supporting Information may be found in the online version of this article.

15273350, 2014, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/hep.26783 by Shanghai Jiaotong University, Wiley Online Library on [25/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

mNOX-E36 has been shown to effectively inhibit macrophage infiltration in murine liver injury models, thereby ameliorating experimental steatohepatitis.[17] Its human equivalent, NOX-E36, is currently being tested in patients with type II diabetes with albuminuria in a phase II interventional study (http://www.ClinicalTrials.gov, NCT01547897).

In this study, we demonstrate the successful pharmacological intervention during regression of murine liver fibrosis. By inhibiting the early influx of Ly-6C$^+$ iMΦ using the CCL2-inhibitor, mNOX-E36, we were able to shift the intrahepatic equilibration toward the "restorative" Ly-6C$^-$ subset of iMΦ in two independent experimental models of regression from toxic and metabolic liver fibrosis. Our data identified a yet unrecognized pathway of transient recruitment of infiltrating Ly-6C$^+$ monocytes during fibrosis regression, which counteract scar resolution by perpetuating inflammatory reactions through release of proinflammatory cytokines such as tumor necrosis factor (TNF).

Materials and Methods

Mice and Liver Injury Models. C57BL/6 (wild type; WT) were housed in a pathogen-free environment. All experiments were performed with male animals at 6-8 weeks of age under ethical conditions approved by the appropriate authorities, according to German legal requirements. Chronic toxic liver injury was induced by CCl_4 (Merck, Darmstadt, Germany), mixed with corn oil, at 0.6 mL/kg body weight (BW) twice-weekly for 6 weeks intraperitoneally (IP), and steatohepatitis was induced by a methionine-choline–deficient (MCD) diet for 8 weeks (catalog no.: 390439; MP Biomedicals, Solon, OH), as previously described.[4,17] For regression studies, injections of CCl_4 were discontinued or mice were switched from MCD to normal chow diet. Liver injury and fibrosis were assessed by biochemical and histological methodology, as described before.[17,19] Gene expression analyses as well as protein and triglyceride (TG) quantifications were performed as detailed earlier.[17,19]

Spiegelmers. The Spiegelmer, mNOX-E36 (50-nucleotide L-RNA oligonucleotide; 5'-GGCGACAUU GGUUGGGCAUGAGGCGAGGCCCUUUGAUGA AUCCGCGGCCA-3') was kindly provided by NOXXON Pharma AG (Berlin, Germany). It binds specifically to murine MCP-1 (CCL2) and inhibits the infiltration of proinflammatory macrophages *in vitro* and *in vivo,* as previously shown.[17,20] The oligonucleotide part of the drug is covalently linked with 40-kDa polyethylene glycol at the 3'-end and was injected subcutaneously (SC) at a dose of 20 mg/kg BW (diluted in 5% glucose solution).[17]

Flow Cytometry and Sorting. Intrahepatic leukocytes were isolated as previously described.[19] Multicolor fluorescence-activated cell sorting (FACS) staining was conducted using combinations of the following mAbs: F4/80 (Serotec); CD115; CD4; CD11c; CD11b (all eBioscience, San Diego, CA); CD45; Gr1/Ly6C; Ly6G; CD19; CD25; NK1.1; CD8; CD206; CD124; and CD3 (all BD Biosciences, San Jose, CA). Dead cells were excluded by Hoechst 33258 dye (Sigma-Aldrich, St. Louis, MO). Analysis was performed using LSR-Fortessa (BD Biosciences), and cell sorting was done by using the FACS Aria-II-SORP (BD Biosciences). Data were analyzed with FlowJo software (TreeStar Inc., Ashalnd, OR). Cytospins from sorted cells were prepared using a Cytospin-II centrifuge (Shandon, Runcorn, UK) and were stained using the DiffQuik-kit (Medion Diagnostics, Düdingen, Switzerland).

Adoptive Transfer of Monocytes. C57BL/6 mice that constitutively expressed red fluorescent protein (RFP) in all cells were used as donors for monocyte transfer experiments. Ly6C^{high} monocytes were isolated from bone marrow (BM) of femurs and tibias by magnetic-assisted cell sorting using biotinylated CD115 mAbs (eBioscience), followed by streptavidin microbeads (Miltenyi Biotec), as described before.[21] Purity of isolated monocytes (>85%) was confirmed by flow cytometry. C57BL/6-recipient mice were injected intravenously with 1-2 $\times$ 10^6 monocytes or phosphate-buffered saline, then received CCl_4 IP and mNOX-E36 or vehicle SC.

BM-Derived Macrophages and* In Vitro *Stimulation. BM cells were isolated from femur and tibia of 8-week-old C57BL/6 mice.[22] To obtain fibroblast-conditioned medium (FCM), L929 fibroblasts were cultured in RPMI medium containing 10% fetal calf serum (FCS) for 3 days, when the cells were still in the growth phase, and the supernatant was collected and stored at −80°C. For the generation of BM-derived macrophages (BMMs), BM cells were cultured in RPMI medium containing 10% FCS and 20% FCM for 1 week on bacterial-grade plastic plates. At day 7, cells were either left untreated or incubated with mNOX-E36 (200 μg/mL). After 24 hours, cells were either left unstimulated or stimulated for an additional 24 hours with recombinant murine interleukin-4 (IL-4; 20 ng/mL) or recombinant murine interferon-gamma (IFN-γ;100 ng/mL; both PeproTech, Rocky Hill, NJ).

Statistical Analysis. All data are presented as mean ± standard deviation (SD). Differences between

15273350, 2014, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/hep.26783 by Shanghai Jiaotong University, Wiley Online Library on [25/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

groups were assessed by the two-tailed unpaired Student *t* test (GraphPad Prism; GraphPad Software, Inc., La Jolla, CA).

Results

Dynamic Changes of Infiltrating Macrophage Subsets During Regression of Chronic Toxic Liver Fibrosis. Macrophage infiltration into the liver upon chronic injury has been convincingly linked to the progression of liver inflammation and fibrosis.[4,5,7,23] More recently, so-called restorative macrophages have been identified that are believed to functionally contribute to the regression of liver fibrosis, likely by degrading matrix proteins by distinct metalloproteinases (MMPs).[1,8,9] When mice were challenged with CCl_4 IP (0.6 mL/kg BW) twice per week for 6 weeks to induce liver fibrosis (Fig. 1A), severe damage with necrotic patches in histology, mononuclear infiltrates, and fibrotic bridging (Fig. 1A) as well as increased aminotransferase activity levels in serum (Fig. 1B) were evident at 48 hours after the last of repetitive CCl_4 injections. At this time point, representing the peak of injury and fibrosis in the chronic toxic model,[4] a massive infiltration of leukocytes, especially macrophages, was detectable by immunohistological staining for the leukocyte marker, CD45, and the macrophage marker, F4/80 (Fig. 1A). As soon as mice were allowed to recover from chronic toxic injury, transaminase levels rapidly decreased to normal values within 5 and 7 days after the last CCl_4 injection (Fig. 1B), whereas inflammatory infiltrates and fibrosis appeared to decline at a slower rate (Fig. 1A).

Recent studies had suggested a predominance of so-called restorative macrophages during fibrosis regression, which was characterized by low to absent expression for the myeloid marker Ly-6C.[1] To characterize the kinetics of intrahepatic macrophages in our model and to address their therapeutic targeting by inhibition of the chemokine CCL2, liver leukocytes were isolated and subjected to multicolor FACS analysis. Intrahepatic iMΦ were defined as living (Hoechst-dye negative) $CD45^+$ cells that stain positive for the myeloid marker CD11b, intermediate for the macrophage marker F4/80, and negative for the neutrophil marker Ly-6G,[17] and were divided in Ly-$6C^+$- and Ly-$6C^-$-expressing subpopulations (Fig. 1D). During fibrosis regression, the total number of hepatic leukocytes declined, mostly resulting from decreasing numbers of iMΦ (Fig. 1C). Strikingly, the relative composition of iMΦ changed significantly during regression, with a progressive increase of the Ly-$6C^+$ iMΦ subset over 7 days, mirrored by decrease in the Ly-$6C^-$ population (Fig. 1E), resulting in a similar Ly-$6C^+$/Ly-$6C^-$ ratio as in nonfibrotic livers of control mice.

To further characterize these subsets of iMΦ during chronic liver damage, we isolated Ly-$6C^+$ and Ly-$6C^-$ $CD11b^+F4/80^+$ hepatic macrophages by FACS sorting. Whereas the Ly-$6C^-$ population represented round-shaped "monocyte-like" cells with small cytoplasm and only few granules (Fig. 2A), the Ly-$6C^+$ iMΦ displayed the typical appearance of "classical" macrophages, including a large, slightly irregularly shaped cell body and scattered granules in the cytoplasm, in line with proinflammatory (secretory and phagocytic) activities (Fig. 2A). These findings were corroborated by gene expression analysis of the subsets, because the Ly-$6C^+$ iMΦ expressed higher levels of TNF, especially in fibrotic livers, compared to the Ly-$6C^-$ fraction or compared to $CD11b^{low}F4/80^{++}$ resident MΦ (Fig. 2B). Interestingly, expression of CCR2 was only slightly enhanced in Ly-$6C^+$ iMΦ (Fig. 2C), contrasting the high CCR2 expression of the Ly-$6C^{high}$ blood monocytes,[24] which are the circulating precursors of the Ly-$6C^+$ iMΦ in inflamed liver.[4] Collectively, these data indicated that Ly-$6C^+$ iMΦ might represent a rather proinflammatory macrophage population during fibrosis regression.

Inhibition of CCL2 Transiently Blocks Ly-$6C^+$ iMΦ During Fibrosis Regression After Toxic Liver Injury. Former studies using *Ccr2*$^{-/-}$ mice demonstrated that chemokine receptor CCR2 and its ligand, CCL2/MCP-1, promote the infiltration of inflammatory Ly-$6C^+$ ($Gr1^+$) monocytes into the liver.[4,12,16] We had previously shown that inhibition of CCL2 by the Spiegelmer mNOX-E36 efficiently blocks infiltration of Ly-$6C^+$ monocytes during fibrosis progression.[17] To investigate the functional role of iMΦ subsets during fibrosis regression, we injected mNOX-E36 (20 mg/kg) SC daily, starting at 24 hours after the last of the repetitive CCl_4 injections (Fig. 3A). Whereas the rapid decline of serum alanine aminotransferase (ALT) levels and the overall numbers of intrahepatic leukocytes were not affected by mNOX-E36 (Fig. 3B,C), FACS analyses of intrahepatic leukocytes revealed a significant reduction of iMΦ upon mNOX-E36 administration at 5 days of regression (Fig. 3D), indicating a continuous and CCL2-dependent influx of (blood-derived) monocytes into regenerating liver during fibrosis regression. Subsequent phenotyping of the iMΦ (Fig. 3E) disclosed a gain of the relative content of Ly-$6C^-$ macrophages, whereas Ly-$6C^+$ iMΦ were reduced (Fig. 3E-F). Interestingly, these effects of CCL2 inhibition on the

15273350, 2014, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/hep.26783 by Shanghai Jiaotong University, Wiley Online Library on [25/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

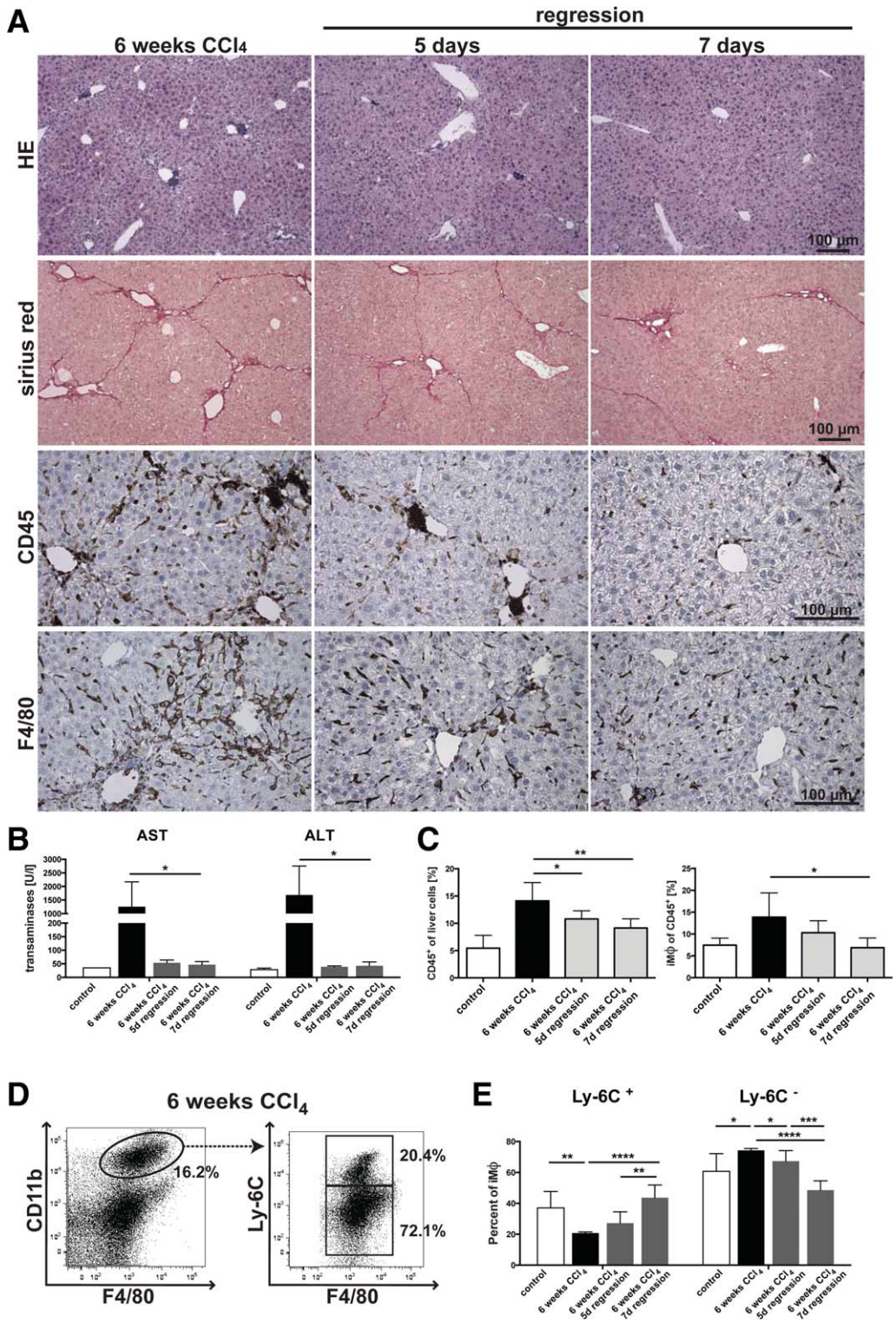

Fig. 1. Dynamic changes of intrahepatic macrophage subsets during regression from chronic toxic liver damage. C57bl/6 WT mice were IP injected with CCl_4 twice-weekly for 6 weeks and analyzed during regression at the indicated time points. (A) Liver histology of paraffin sections showed necrosis and progressive mononuclear infiltrates in the periportal regions of chronic injured livers from WT mice (hematoxylin-eosin stainings; HE), collagen deposition (Sirius Red stainings), and leukocyte and macrophage infiltration (identified by immunohistochemistry for CD45 and F4/80, respectively). (B) Hepatic injury was also assessed by serum AST and ALT levels. (C) Infiltration of leukocytes (percentage of $CD45^+$ cells from total liver cells) and infiltrating macrophages (iMΦ; Hoechst-dye$^-$/$CD45^+$/Ly-6G$^-$/$CD11b^+$/$F4/80^+$) into the liver was quantified by FACS, revealing decreasing numbers of iMΦ during regression. (D and E) FACS analyses of intrahepatic iMΦ subsets (pregated as described) showing elevated numbers of Ly-$6C^+$ and reduced numbers of Ly-$6C^-$ intrahepatic macrophages after 7 days of regression. All data are expressed as mean ± SD, comprising n = 9 animals in each group. *$P < 0.05$; **$P < 0.005$; ***$P < 0.001$; ****$P < 0.0001$ (unpaired Student *t* test).

15273350, 2014, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/hep.26783 by Shanghai Jiaotong University, Wiley Online Library on [25/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

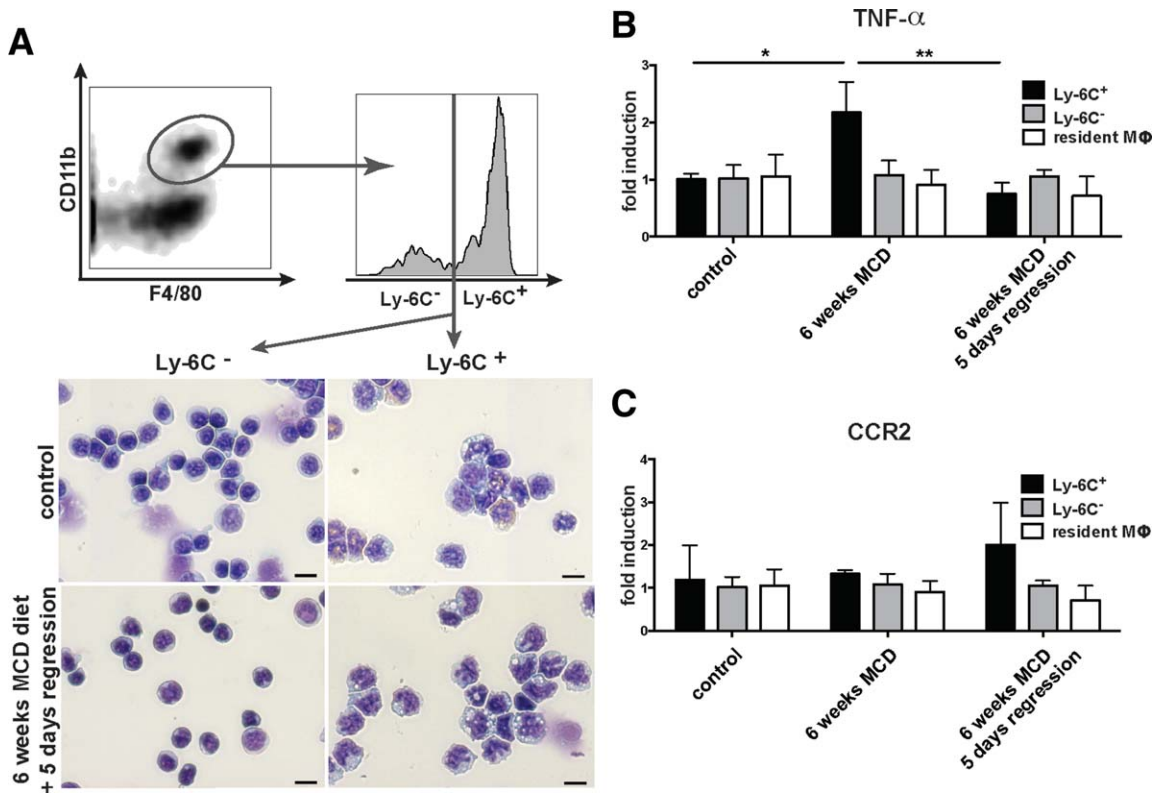

Fig. 2. Morphology and phenotype of intrahepatic macrophage subsets during regression from chronic liver damage. (A) C57bl/6 WT mice were fed by an MCD diet for 6 weeks to induce chronic steatohepatitis and analyzed after 5 days of regression. Cytospins from sorted Ly-6C$^+$ and Ly-6C$^-$ cells of iMΦ revealed the morphology of metabolically active macrophages in the Ly-6C$^+$ population and a more "monocyte-like" appearance in the Ly-6C$^-$ subset. (B and C) Gene expression analysis from sorted Ly-6C$^+$, Ly-6C$^-$, and resident intrahepatic macrophages (MΦ, CD11b^{low}F4/80^{++}) by qPCR showed elevated TNF-α levels in Ly-6C$^+$ cells, especially in fibrotic livers. All data are expressed as mean ± SD, comprising n = 5 for animals in the treatment and n = 4 animals in the control groups. *$P < 0.05$; **$P < 0.005$; ***$P < 0.001$; ****$P < 0.0001$ (unpaired Student *t* test).

composition of hepatic iMΦ was observed only at 5 days of regression, suggesting that CCL2-dependent recruitment of Ly-6C$^+$ monocytes is a transient event relatively early during regression from liver fibrosis (Fig. 3D-F). Importantly, these effects were specific to hepatic macrophages, because other intrahepatic leukocyte subpopulations, such as T (CD8$^+$, CD4$^+$) or natural killer (NK) cells were not affected by mNOX-E36 administration (Supporting Fig. 1).

CCL2-Dependent Variations of iMΦ Subsets During Regression of Chronic Metabolic Liver Injury. To exclude that model-specific effects had confounded the above-mentioned results, we employed a second model of chronic liver injury. Mice were fed an MCD diet for 8 weeks (Fig. 4A), resulting in severe steatohepatitis at a macroscopic and microscopic level (Fig. 4B) as well as elevated ALT (Fig. 4C) and aspartase aminotransferase (AST; not shown) levels.[17] When mice were switched to a normal chow diet to induce regression from steatohepatitis, liver injury (histology and ALT) was significantly reduced after 5 days (Fig. 4B,C), alongside a significant reduction in total hepatic leukocytes (Fig. 4D) and intrahepatic CD11b$^+$F4/80$^+$ iMΦ (Fig. 4D). Inhibition of MCP-1/CCL2 by mNOX-E36 further reduced hepatic leukocyte and iMΦ counts during regression (Fig. 4B,D). Moreover, in line with the results from the CCl_4 injury model, the composition of intrahepatic macrophages was altered by mNOX-E36 (Fig. 4E), resulting in a reduced proportion of Ly-6C$^+$ and increased proportion of Ly-6C$^-$ iMΦ (Fig. 4F). Again, other intrahepatic leukocyte populations, such as T (CD8$^+$ and CD4$^+$) or NK cells were not affected by mNOX-E36 administration during the regression phase in the MCD diet model (Supporting Fig. 2A-E). Collectively, these data indicated that Ly-6C$^+$ monocytes transiently accumulate in the liver in both models of regression from chronic liver injury, which can be blocked by pharmacological inhibition of the chemokine MCP-1/CCL2.

Fibrosis Regression Is Accelerated in mNOX-E36-Treated Mice After Toxic and Metabolic Chronic Liver Injury. We hypothesized that pharmacological inhibition of CCL2 could represent a successful

15273350, 2014, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/hep.26783 by Shanghai Jiaotong University, Wiley Online Library on [25/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

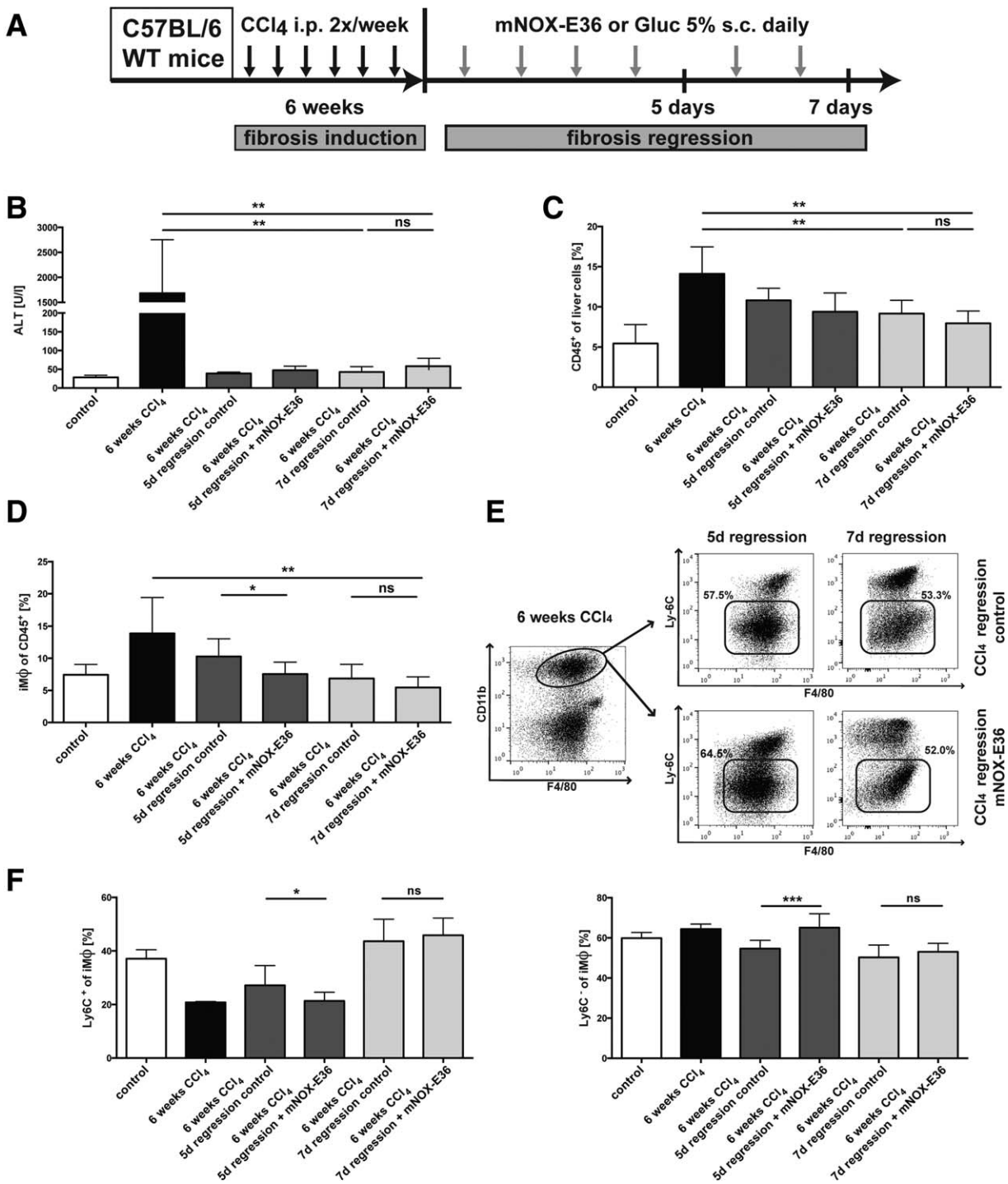

Fig. 3. mNOX-E36 inhibits infiltration of Ly-6C$^+$ iMΦ during fibrosis regression after toxic liver injury. (A) C57bl/6 WT mice were IP injected with CCl_4 twice-weekly for 6 weeks and analyzed during regression with or without additional SC administration of mNOX-E36 (or vehicle [glucose 5%] control) at the indicated time points. (B) Hepatic injury was assessed by serum ALT levels. (C-D) Infiltration of leukocytes (CD45$^+$) and iMΦ (gated as described before) into the liver was quantified by FACS, revealing decreased numbers of iMΦ upon mNOX-E36 administration after 5 days. (E and F) FACS analyses of intrahepatic iMΦ subsets (Ly-6C$^{+/-}$) from mice treated with mNOX-E36 showing reduced numbers of Ly-6C$^+$ and elevated numbers of Ly-6C$^-$ intrahepatic macrophages after 5 days of regression. All data are expressed as mean ± SD, comprising n = 12 for animals in the treatment and n = 5-7 animals in the control groups. *$P < 0.05$; **$P < 0.005$; ***$P < 0.001$; ****$P < 0.0001$ (unpaired Student *t* test).

15273350, 2014, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/hep.26783 by Shanghai Jiaotong University, Wiley Online Library on [25/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

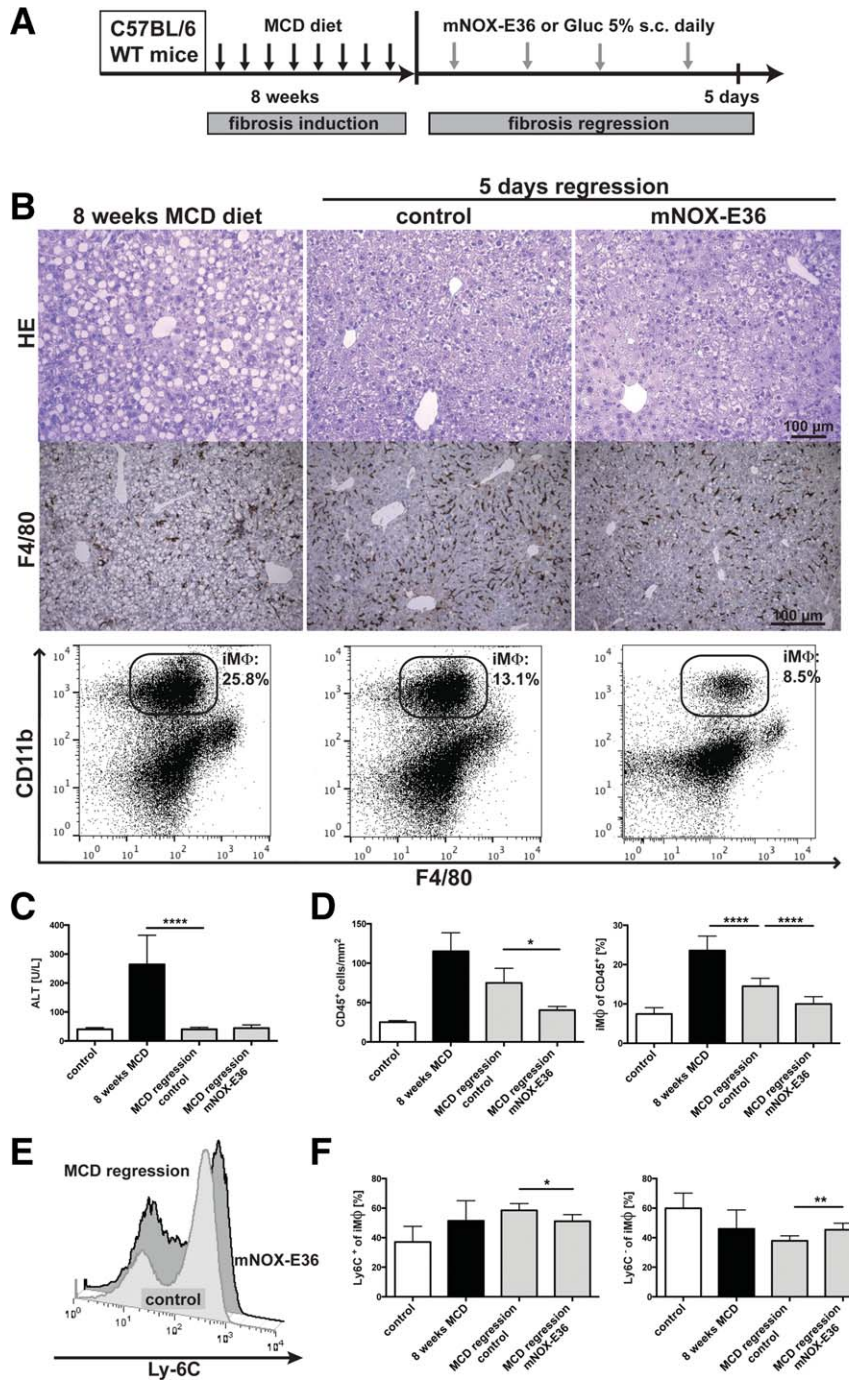

Fig. 4. mNOX-E36 inhibits infiltration of Ly-6C$^+$ iMΦ during fibrosis regression after metabolic liver injury. (A) C57bl/6 WT mice were fed by an MCD diet for 8 weeks to induce steatohepatitis as well as fibrosis and analyzed during regression with or without mNOX-E36 or tracer control treatment at the indicated time points. (B) Liver histology of paraffin sections showed attenuated mononuclear infiltrates in the periportal regions of steatotic livers from mNOX-E36-treated mice (hematoxylin-eosin stainings; HE) and attenuated macrophage infiltration (identified by immunohistochemistry for F4/80 and FACS analysis). (C) Hepatic injury was assessed by ALT levels. (D) Infiltration of leukocytes (CD45$^+$) and iMΦ (gated as described before) into the liver was quantified by counting of CD45$^+$ cells on immunohistologically stained liver sections and FACS analyses, revealing decreased numbers of iMΦ upon mNOX-E36 administration after 5 days. (E and F) FACS analyses of intrahepatic iMΦ subsets (Ly-6C$^{+/-}$) from mice treated with mNOX-E36 showing reduced numbers of Ly-6C$^+$ and elevated numbers of Ly-6C$^-$ intrahepatic macrophages upon mNOX-E36 treatment after 5 days of regression. All data are expressed as mean ± SD, comprising n = 10-12 for animals in the regression and n = 5-7 animals in the control groups. *$P < 0.05$; **$P < 0.005$; ***$P < 0.001$; ****$P < 0.0001$ (unpaired Student *t* test).

therapeutic approach to enhance fibrosis regression in chronic liver injury *in vivo* by blocking Ly-6C$^+$ monocyte infiltration and shifting the iMΦ population toward the Ly-6C$^-$ phenotype. Indeed, fibrotic degeneration of the liver was significantly attenuated in mice with chronic CCl_4-induced toxic liver damage after 7 days of regression upon mNOX-E36 administration, as evidenced by reduced collagen deposition in histology, hydroxyproline content, and Sirius Red–positive areas (Fig. 5A). Similar findings were observed during recovery from metabolic MCD diet-induced liver injury. Here, also a significant reduction of the content of hydroxyproline and lower levels of Sirius Red–positive areas were detected when CCL2 was inhibited by mNOX-E36 (Fig. 5B,C). Hepatic TG content as a measure for steatosis was not further decreased in mNOX-E36-treated animals in the MCD diet regression model (Fig. 5D and Supporting Fig. 3A,B), indicating specific effects of the iMΦ subsets in controlling fibrosis regression. In line with the histological and biochemical data on fibrosis regression, quantitative expression analyses of fibrosis-related genes confirmed

15273350, 2014, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/hep.26783 by Shanghai Jiaotong University, Wiley Online Library on [25/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

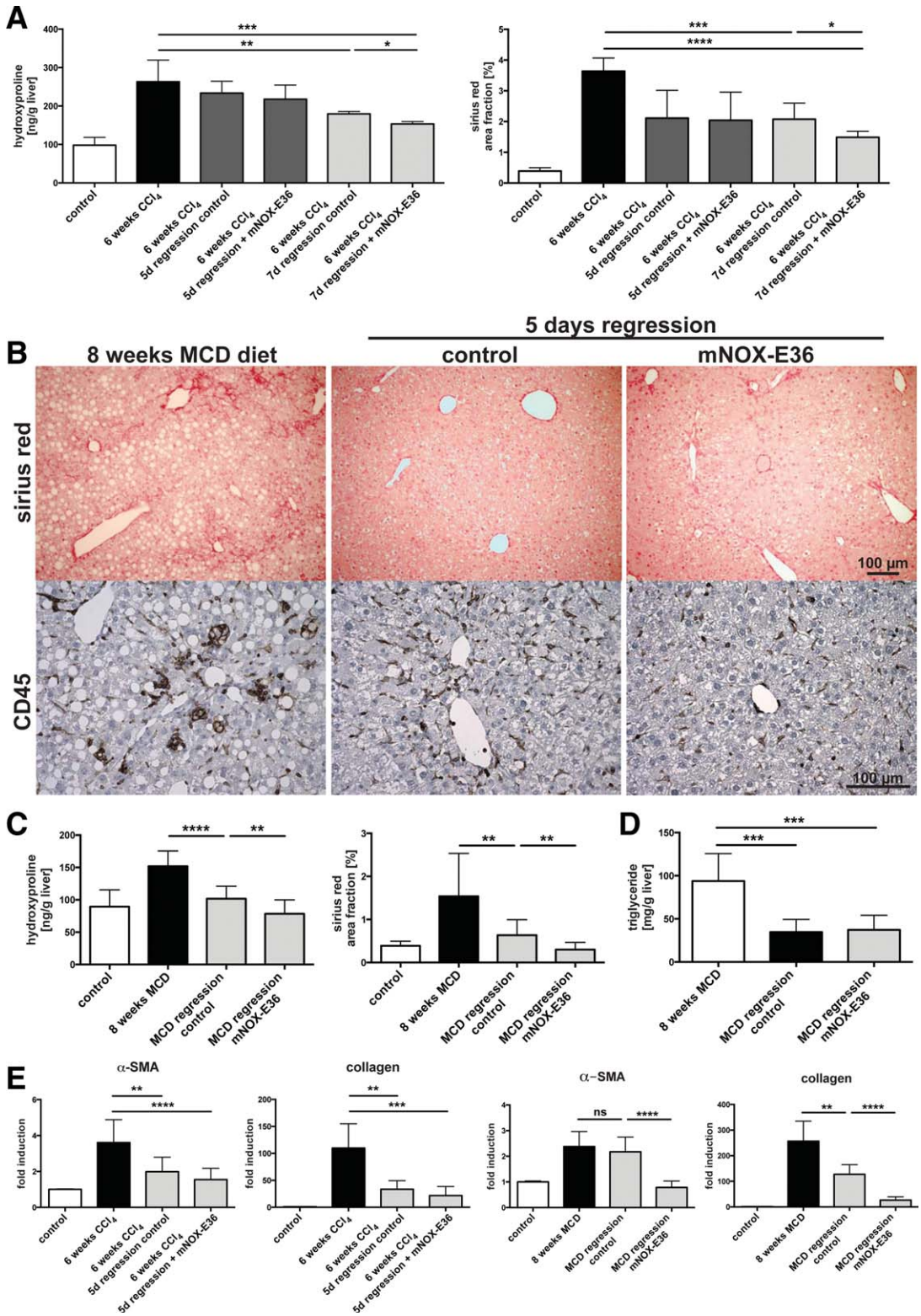

Fig. 5. mNOX-E36-treated mice show accelerated regression from liver fibrosis induced by CCl_4 or MCD diet. Liver fibrosis was induced in C57bl/6 WT mice, and mNOX-E36 was administered as described. (A) Collagen deposition, as quantified by intrahepatic hydroxyproline measurement and Sirius Red stainings, was significantly decreased in mNOX-E36-treated mice after 7 days of regression from toxic liver fibrosis. (B) Liver histology of paraffin sections showed attenuated $CD45^+$ infiltrates in the periportal regions of steatotic livers from mNOX-E36-treated mice (immunohistochemistry for CD45) and reduced collagen deposition (Sirius Red stainings) during regression from MCD diet. (C) Collagen deposition, as quantified by intrahepatic hydroxyproline measurement and Sirius Red stainings, was significantly decreased in mNOX-E36-treated mice mice after 5 days of regression from metabolic liver fibrosis. (D) Quantification of TGs from total liver lysates revealed no difference in murine liver with or without mNOX-E36 treatment. (E) Gene expression analyses from whole liver by qPCR showed reduced hepatic α-SMA and collagen mRNA levels in mNOX-E36-injected mice. All data are expressed as mean ± SD, comprising n = 10-12 for animals in the treatment and n = 5-7 animals in the control groups. $*P < 0.05$; $**P < 0.005$; $***P < 0.001$; $****P < 0.0001$ (unpaired Student t test).

15273350, 2014, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/hep.26783 by Shanghai Jiaotong University, Wiley Online Library on [25/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

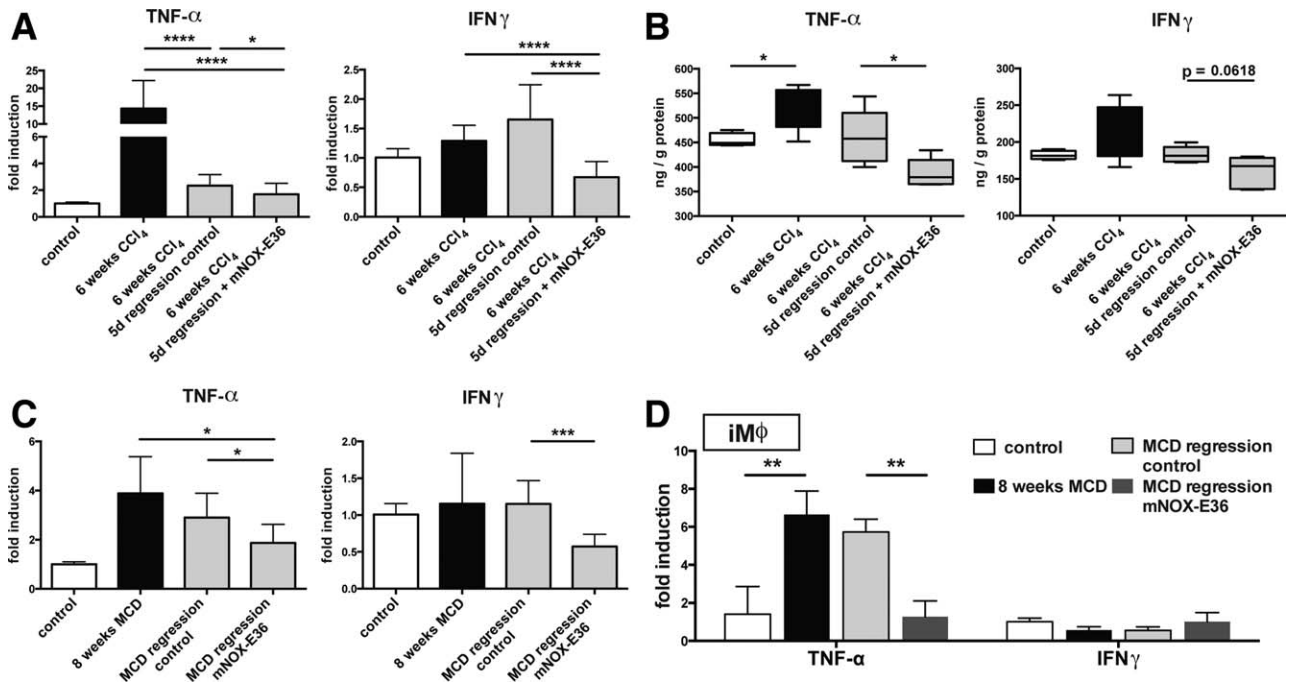

Fig. 6. Intrahepatic cytokine alterations upon mNOX-E36 administration *in vivo* and *in vitro*. Liver fibrosis was induced in C57bl/6 WT mice, and mNOX-E36 was admitted as described. (A-C) Gene and protein expression analysis from whole liver by qPCR and ELISA from whole-liver lysates showed reduced hepatic TNF-α and IFN-γ levels in mNOX-E36-injected mice in regression from CCl_4- (A and B) and MCD-diet–induced (C) fibrosis. (D) Gene expression analysis from FACS iMΦ showed reduced TNF-α mRNA levels in mNOX-E36-injected mice at regression from metabolic fibrosis. All data are expressed as mean ± SD, comprising n = 6-12 animals for intrahepatic gene analyses and cytokine measurements and n = 4 for gene analysis of FACS iMΦ. $*P < 0.05$; $**P < 0.005$; $***P < 0.001$; $****P < 0.0001$ (unpaired Student *t* test).

reduction of alpha-smooth muscle actin (α-SMA) and collagen messenger RNA (mRNA) in liver samples of mice treated with mNOX-E36 during regression (Fig. 5E). Of note, CCL2 serum levels were elevated in the MCD diet, as well as in CCl_4-induced liver fibrosis, and moderately declined during fibrosis regression (Supporting Fig. 3C). Administration of mNOX-E36 resulted in massively up-regulated CCL2 serum levels; this is explained by the fact that mNOX-E36 blocks the biologic activity, but not the detectability by enzyme-linked immunosorbent assay (ELISA), of the CCL2 protein. Thus, these data corroborate the strong inhibitory effects of mNOX-E36 on CCL2 *in vivo* (Supporting Fig. 3C).

Pharmacological Inhibition of CCL2-Dependent Ly-6C$^+$ iMΦ Reduces Intrahepatic Proinflammatory Cytokines During Fibrosis Regression. An important function of hepatic macrophages during disease progression and regression is the secretion of proinflammatory cytokines.[17,23,25] To explore potential mechanisms how blocking of Ly-6C$^+$ iMΦs by mNOX-E36 accelerates fibrosis regression, intrahepatic cytokine levels were measured. During regression from CCl_4-induced fibrosis, expression of TNF-α and IFN-γ was significantly down-regulated in livers of mice that received mNOX-E36, both on a gene (Fig. 6A) and protein (Fig. 6B) level. Similar findings were confirmed during regression from MCD diet injury by quantitative polymerase chain reaction (qPCR; Fig. 6C) and ELISA (data not shown).

To link the intrahepatic cytokine profile to distinct mononuclear cell populations in the liver, primary hepatic iMΦ were isolated from livers of mice by FACS (populations as depicted in Fig. 4B).[19] After mNOX-E36 treatment, iMΦ of mice that received the MCD diet showed decreased expression levels of TNF-α, whereas IFN-γ synthesis was unaffected (Fig. 6D). Of note, TNF-α expression in simultaneously isolated resident macrophages, so-called KCs (F4/80^{++}CD11b^{low}), remained unaffected by mNOX-E36 treatment (Fig. 2B). Subset analysis of Ly-6C$^+$ and Ly-6C$^-$ macrophages disclosed increased TNF-α production in Ly-6C$^+$-expressing cells (Fig. 2B), indicating that these cells are the major TNF-α−producing macrophage population within the injured liver.

To exclude that the CCL2 inhibitor, mNOX-E36, itself had direct or indirect (e.g., by interfering with autocrine CCL2 pathways) effects on TNF-α expression, BM-derived macrophages were generated from WT mice, cultured with or without the presence of mNOX-E36, and stimulated with either IFN-γ (to

15273350, 2014, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/hep.26783 by Shanghai Jiaotong University, Wiley Online Library on [25/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

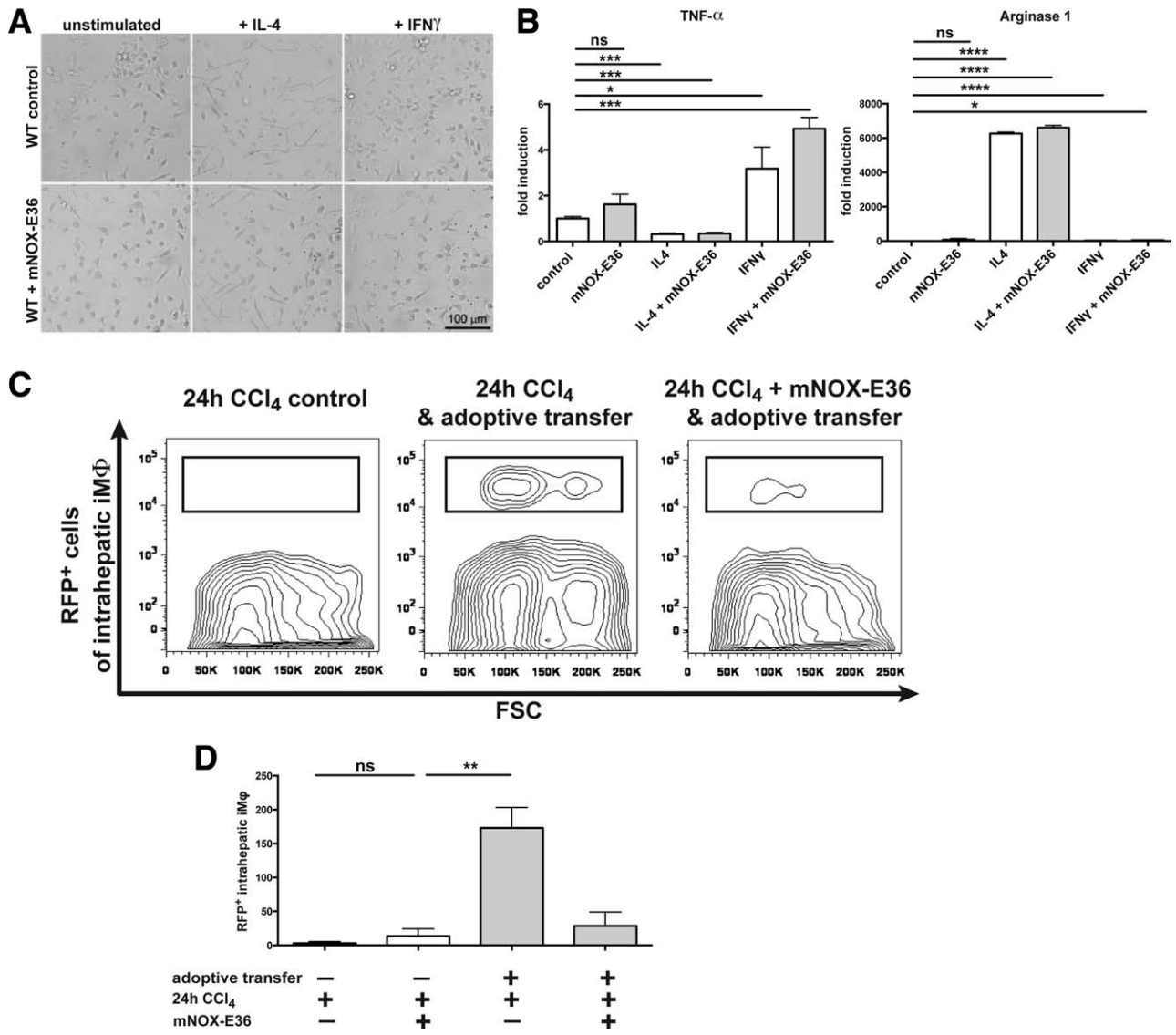

Fig. 7. Infiltration of Ly-6C$^+$ macrophages, but not macrophage polarization, is blocked by mNOX-E36. (A) BMMs were isolated and cultured with or without mNOX-E36, followed by stimulation with IL-4 (M2 polarization) or IFN-γ (M1 polarization). (B) Gene expression analysis by qPCR for TNF-α (M1 effector cytokine) and arginase 1 (M2 marker). (C and D) Adoptive transfer of Ly-6C$^+$ monocytes isolated from BM of RFP-expressing mice displayed infiltration of RFP$^+$ Ly-6C$^+$ monocytes 24 hours after CCl_4 injury, which could be effectively blocked by a single administration of mNOX-E36. All data are expressed as mean $\pm$ SD, comprising n = 12 for animals in the treatment and n = 5-7 animals in the control groups. *$P < 0.05$; **$P < 0.005$; ***$P < 0.001$; ****$P < 0.0001$ (unpaired Student *t* test).

induce M1 polarization) or IL-4 (to induce M2 polarization; Fig. 7A). Neither the baseline nor the IFN-γ-induced TNF-α expression were suppressed by mNOX-E36 (Fig. 7B). Of note, mNOX-E36 treatment on macrophages did not induce or facilitate IL4-induced M2 polarization either, as assessed by arginase expression (Fig. 7B). Furthermore, we wanted to ensure that mNOX-E36 specifically inhibits Ly-6C$^+$ monocyte migration from the circulation into the liver and exclude that the changes in intrahepatic macrophage subsets are a secondary effect of accelerated fibrosis regression induced by mNOX-E36 by alternative pathways. Therefore, Ly-6C$^+$ monocytes were isolated from BM of RFP Tg mice and adoptively transferred into mice challenged with CCl_4. Twenty-four hours after injury induction, a clear hepatic infiltration of RFP$^+$ monocytes was revealed (Fig. 7C). This intrahepatic Ly-6C$^+$ monocyte accumulation could be almost completely blocked (to background levels) by a single injection of mNOX-E36 (Fig. 7C,D). Moreover, adoptively transferred RFP$^+$ Ly-6C$^+$ monocytes recovered from liver displayed similar Ly-6C expression levels as endogenous intrahepatic Ly-6C$^+$ iMΦs, corroborating that mNOX-E36 did not simply induce a rapid down-regulation of Ly-6C expression (detailed data not shown).

15273350, 2014, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/hep.26783 by Shanghai Jiaotong University, Wiley Online Library on [25/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Discussion

Organ fibrosis and cirrhosis is the common end-stage disease of the liver with life-threatening complications such as gastrointestinal bleeding and hepatocellular carcinoma.[26] In humans, the only available curative treatment for end-stage liver disease is the transplantation of the liver, emphasizing the urgent need to find new therapeutic strategies for liver cirrhosis. Based on a growing body of experimental evidence on the crucial function of monocytes and macrophages for fibrosis modulation, these cells might represent attractive novel targets for antifibrotic strategies.[27] Earlier studies investigated to date the potential role of BM cell therapy in liver fibrosis,[7,28] but the effects were rather limited and the procedure is complex.[29] Here, especially unstimulated BMMs, which neither possess the typical classical (M1) nor the alternatively activated (M2) gene profile, achieved good results in preventing experimental liver fibrosis in mice.[10] It has also been shown that the administration of distinct macrophage subsets, especially subpopulations with low Ly-6C expression, can improve liver fibrosis during regression.[10] On the other hand, adoptive transfer of immature Ly-6C$^+$ BM-derived monocytes aggravates experimental liver fibrosis.[4]

Besides transferring distinct populations of myeloid cells, influencing the balance between "inflammatory" and "restorative" macrophages might bear therapeutic potential. In this study, we demonstrate that a novel compound for specific CCL2 inhibition, the Spiegelmer mNOX-E36, effectively inhibits intrahepatic accumulation of Ly-6C$^+$ iMΦ during regression from different chronic liver injury models. Hereby, mNOX-E36 augmented the proportion of restorative Ly-6C$^-$ macrophages, which finally led to accelerated regression from liver fibrosis after toxic or metabolic liver injury.

Several independent studies highlighted the importance of the chemokine receptor CCR2 and its main ligand, MCP-1/CCL2, for monocyte/macrophage recruitment during experimental hepatic fibrosis,[4,12,15-17] suggesting that inhibition of CCR2 or CCL2 might bear therapeutic potential in chronic liver diseases. Nevertheless, inhibiting CCL2 during fibrosis *progression* with mNOX-E36 efficiently ameliorated steatohepatitis, but did not alleviate fibrosis development to a relevant extent in a previous study.[17] In contrary, our data clearly demonstrate that CCL2 inhibition was efficient in accelerating *regression* from hepatic fibrosis in two independent experimental models.

Thus, our findings highlight the specific relevance of the (chemokine-dependent) accumulation and differentiation of monocyte/macrophage subpopulations during the regression of liver fibrosis. The beneficial actions of Ly-6C$^-$ restorative macrophages have been demonstrated for CCl_4-induced fibrosis before and were linked to expression of MMPs, growth factors, and phagocytosis-related genes.[1] However, it has been proposed that these restorative macrophages mainly originate from Ly-6C$^+$ monocytes that freshly infiltrate the liver during regression of fibrosis.[1] The fact that inhibition of CCL2-dependent accumulation of Ly-6C$^+$ monocytes in regressing hepatic fibrosis effectively accelerates fibrosis regression indicates that these cells might rather act as a double-edged sword in fibrosis regression. Although Ly-6C$^+$ monocytes might be capable of giving rise to restorative Ly-6C$^-$ macrophages, their inhibition results in a relative increase of either already existing Ly-6C$^-$ macrophages in the liver or recruitment of such cells from other precursors (e.g., from Ly-6C$^-$ monocytes).[30] Importantly, our *in vitro* experiments with BMMs could exclude that the CCL2 inhibitor itself had a direct effect on macrophage cytokine release or their M1/M2 polarization. From a functional point of view, mNOX-E36 administration significantly reduced the levels of proinflammatory cytokines (TNF-α and IFN-γ) during disease regression in the liver, in line with the reduced macrophage numbers. These results clearly assign a "proinflammatory" profile to Ly-6C$^+$ iMΦ, in line with a typical morphology of inflammatory macrophages and TNF expression upon isolation from injured liver.[3] Although our results indicate that Ly-6C$^+$ iMΦ are the main source of TNF in the injured liver, further studies are needed to confirm cytokine expression of distinct macrophage subsets on protein level, as well as further dissect to which extent these hepatic macrophage populations may produce or release TNF upon different stimuli (Toll-like receptor 4 agonists, apoptotic hepatocytes, or others). Overall, the perpetuation of inflammation, as reflected by proinflammatory cytokine levels in the liver, appears to counteract regenerative processes and degradation of ECM, thus inhibiting resolution of fibrosis.[31]

Translating the findings from murine models into human pathogenesis is hampered by the fact that murine and human monocyte/macrophage subpopulations do not share the same surface-marker molecules.[32] For instance, murine monocytes and macrophages are distinguished by different levels of Ly-6C (Gr1), whereas CD14 and CD16 are typical markers for corresponding subsets in humans.[33] In fact, the exact human counterpart of restorative

15273350, 2014, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/hep.26783 by Shanghai Jiaotong University, Wiley Online Library on [25/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

macrophages, being Ly-6C$^-$ in mice, still remains to be clarified. On the contrary, the recruitment from blood and the proinflammatory expression pattern of Ly-6C$^+$ monocytes and macrophages observed during the regression phase of fibrosis and effectively blocked by mNOX-E36 in murine models are very similar to the phenotype of CD14^{++}CD16$^+$ "intermediate" monocytes in human liver diseases,[5,6] giving rise to the expectation that application of an CCL2 inhibitor in human fibrosis regression holds significant therapeutic potential.

Acknowledgment: The authors thank Aline Roggenkamp, Carmen Tag, and Sibille Sauer-Lehnen for their excellent technical assistance and the chemistry group of NOXXON Pharma AG (Berlin, Germany) for providing mNOX-E36.

References

1. Ramachandran P, Pellicoro A, Vernon MA, Boulter L, Aucott RL, Ali A, et al. Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. Proc Natl Acad Sci U S A 2012;109:E3186-E3195.
2. Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, et al. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet 2013;381:468-475.
3. Heymann F, Trautwein C, Tacke F. Monocytes and macrophages as cellular targets in liver fibrosis. Inflamm Allergy Drug Targets 2009;8:307-318.
4. Karlmark KR, Weiskirchen R, Zimmermann HW, Gassler N, Ginhoux F, Weber C, et al. Hepatic recruitment of the inflammatory Gr1+ monocyte subset upon liver injury promotes hepatic fibrosis. Hepatology 2009;50:261-274.
5. Zimmermann HW, Seidler S, Nattermann J, Gassler N, Hellerbrand C, Zernecke A, et al. Functional contribution of elevated circulating and hepatic non-classical CD14CD16 monocytes to inflammation and human liver fibrosis. PLoS One 2010;5:e11049.
6. Liaskou E, Zimmermann HW, Li KK, Oo YH, Suresh S, Stamataki Z, et al. Monocyte subsets in human liver disease show distinct phenotypic and functional characteristics. Hepatology 2013;57:385-398.
7. Duffield JS, Forbes SJ, Constandinou CM, Clay S, Partolina M, Vuthoori S, et al. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J Clin Invest 2005;115:56-65.
8. Fallowfield JA, Mizuno M, Kendall TJ, Constandinou CM, Benyon RC, Duffield JS, Iredale JP. Scar-associated macrophages are a major source of hepatic matrix metalloproteinase-13 and facilitate the resolution of murine hepatic fibrosis. J Immunol 2007;178:5288-5295.
9. Pellicoro A, Aucott RL, Ramachandran P, Robson AJ, Fallowfield JA, Snowdon VK, et al. Elastin accumulation is regulated at the level of degradation by macrophage metalloelastase (MMP-12) during experimental liver fibrosis. Hepatology 2012;55:1965-1975.
10. Thomas JA, Pope C, Wojtacha D, Robson AJ, Gordon-Walker TT, Hartland S, et al. Macrophage therapy for murine liver fibrosis recruits host effector cells improving fibrosis, regeneration, and function. Hepatology 2011;53:2003-2015.
11. Jiao J, Sastre D, Fiel MI, Lee UE, Ghiassi-Nejad Z, Ginhoux F, et al. Dendritic cell regulation of carbon tetrachloride-induced murine liver fibrosis regression. Hepatology 2012;55:244-255.
12. Seki E, de Minicis S, Inokuchi S, Taura K, Miyai K, van Rooijen N, et al. CCR2 promotes hepatic fibrosis in mice. Hepatology 2009;50:185-197.
13. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res 2009;29:313-326.
14. Marra F, DeFranco R, Grappone C, Milani S, Pastacaldi S, Pinzani M, et al. Increased expression of monocyte chemotactic protein-1 during active hepatic fibrogenesis: correlation with monocyte infiltration. Am J Pathol 1998;152:423-430.
15. Galastri S, Zamara E, Milani S, Novo E, Provenzano A, Delogu W, et al. Lack of CC chemokine ligand 2 differentially affects inflammation and fibrosis according to the genetic background in a murine model of steatohepatitis. Clin Sci (Lond) 2012;123:459-471.
16. Mitchell C, Couton D, Couty JP, Anson M, Crain AM, Bizet V, et al. Dual role of CCR2 in the constitution and the resolution of liver fibrosis in mice. Am J Pathol 2009;174:1766-1775.
17. Baeck C, Wehr A, Karlmark KR, Heymann F, Vucur M, Gassler N, et al. Pharmacological inhibition of the chemokine CCL2 (MCP-1) diminishes liver macrophage infiltration and steatohepatitis in chronic hepatic injury. Gut 2012;61:416-426.
18. Wlotzka B, Leva S, Eschgfaller B, Burmeister J, Kleinjung F, Kaduk C, et al. In vivo properties of an anti-GnRH Spiegelmer: an example of an oligonucleotide-based therapeutic substance class. Proc Natl Acad Sci U S A 2002;99:8898-8902.
19. Heymann F, Hammerich L, Storch D, Bartneck M, Huss S, Russeler V, et al. Hepatic macrophage migration and differentiation critical for liver fibrosis is mediated by the chemokine receptor C-C motif chemokine receptor 8 in mice. Hepatology 2012;55:898-909.
20. Ninichuk V, Clauss S, Kulkarni O, Schmid H, Segerer S, Radomska E, et al. Late onset of Ccl2 blockade with the Spiegelmer mNOX-E36-3'PEG prevents glomerulosclerosis and improves glomerular filtration rate in db/db mice. Am J Pathol 2008;172:628-637.
21. Ginhoux F, Tacke F, Angeli V, Bogunovic M, Loubeau M, Dai XM, et al. Langerhans cells arise from monocytes in vivo. Nat Immunol 2006;7:265-273.
22. Tacke F, Ginhoux F, Jakubzick C, van Rooijen N, Merad M, Randolph GJ. Immature monocytes acquire antigens from other cells in the bone marrow and present them to T cells after maturing in the periphery. J Exp Med 2006;203:583-597.
23. Karlmark KR, Zimmermann HW, Roderburg C, Gassler N, Wasmuth HE, Luedde T, et al. The fractalkine receptor CX(3)CR1 protects against liver fibrosis by controlling differentiation and survival of infiltrating hepatic monocytes. Hepatology 2010;52:1769-1782.
24. Tacke F, Alvarez D, Kaplan TJ, Jakubzick C, Spanbroek R, Llodra J, et al. Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques. J Clin Invest 2007;117:185-194.
25. Zimmermann HW, Trautwein C, Tacke F. Functional role of monocytes and macrophages for the inflammatory response in acute liver injury. Front Physiol 2012;3:56.
26. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005;115:209-218.
27. Zimmermann HW, Tacke F. Modification of chemokine pathways and immune cell infiltration as a novel therapeutic approach in liver inflammation and fibrosis. Inflamm Allergy Drug Targets 2011;10:509-536.
28. Forbes SJ, Russo FP, Rey V, Burra P, Rugge M, Wright NA, Alison MR. A significant proportion of myofibroblasts are of bone marrow origin in human liver fibrosis. Gastroenterology 2004;126:955-963.
29. Houlihan DD, Newsome PN. Critical review of clinical trials of bone marrow stem cells in liver disease. Gastroenterology 2008;135:438-450.
30. Hanna RN, Carlin LM, Hubbeling HG, Nackiewicz D, Green AM, Punt JA, et al. The transcription factor NR4A1 (Nur77) controls bone marrow differentiation and the survival of Ly6C- monocytes. Nat Immunol 2011;12:778-785.
31. Stauffer JK, Scarzello AJ, Jiang Q, Wiltrout RH. Chronic inflammation, immune escape, and oncogenesis in the liver: a unique neighborhood for novel intersections. Hepatology 2012;56:1567-1574.
32. Ingersoll MA, Spanbroek R, Lottaz C, Gautier EL, Frankenberger M, Hoffmann R, et al. Comparison of gene expression profiles between human and mouse monocyte subsets. Blood 2010;115:e10-e19.
33. Tacke F. Functional role of intrahepatic monocyte subsets for the progression of liver inflammation and liver fibrosis in vivo. Fibrogenesis Tissue Repair 2012;5(Suppl 1):S27.

15273350, 2014, 3, Downloaded from https://onlinelibrary.wiley.com/doi/10.1002/hep.26783 by Shanghai Jiaotong University, Wiley Online Library on [25/10/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License